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The fermentation efficiency exhibited by *Saccharomyces cerevisiae* on Sugarcane bagasse hydrolysate, by analyzing the effects of pre-treatment and detoxification

A eficiência da fermentação exibida por *Saccharomyces cerevisiae* no hidrolisado de bagaço de cana-de-açúcar, analisando os efeitos do pré-tratamento e destoxicação

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Highlights

Hydrothermal and acidic pre-treatment removed a high fraction of hemicellulose.
Furfural and acetic acid exhibited a toxic effect on *S. cerevisiae* and inhibitors the fermentation.
Increase in concentration of *S. cerevisiae* decreased their sensitivity to the inhibitor's.
The detoxification process increased fermentation efficiency of *S. cerevisiae.*

Abstract

In this study, the possibility of increasing fermentation efficiency of *Saccharomyces cerevisiae* on sugarcane bagasse (a type of lignocellulosic waste) was analyzed. Sugarcane bagasse was subjected to hydrothermal and acidic pre-treatment. Next, the enzymatic hydrolysis of raw biomass and each pre-

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treated biomass was performed using CellicCtec® enzymatic complex to obtain sugarcane hydrolysate, hydrothermal hydrolysate and acidic hydrolysate. Next, these were fermented by S. cerevisiae to check if the by-products of enzymatic hydrolysis, furfural and acetic acid had an inhibitory effect on fermentation efficiency. Next, each pre-treated biomass was subjected to detoxification involving activated charcoal. Each detoxified biomass was tested for fermentation efficiency. The lignocellulosic composition for sugarcane hydrolysate, hydrothermal hydrolysate and acidic hydrolysate, varied significantly, and were found to be, for cellulose 36.7%, 27.7% and 63.7% respectively; for hemicellulose 22.2%, 4.4% and 12% respectively; and for lignin 21.2%, 27.7% and 28.7% respectively. The presence of furfural and acetic acid had a strong influence on the fermentation efficiency of S. cerevisiae, and affected the consumption of sugars in each biomass by more than 90%. Further, we found that the detoxification process increased fermentation efficiency by 12.7% for the hydrothermal hydrolysate while for the acidic hydrolysate no significant difference was observed. This study showed that fermentation with greater efficiency is viable through the combined use of hydrothermal pre-treatment and detoxification. This combination of methods also causes less pollution as compared with the method involving acid pre-treatment due to the reduced number of effluents produced.
Key words: Lignocellulosic feedstock. Inhibitors. Ethanol. Degradation products.

Resumo

Nesse trabalho avaliou-se a possibilidade de se aumentar a eficiência de fermentação de um hidrolisado de bagaço de cana submetido aos pré-tratamentos hidrotérmico (195 ºC, usando 200 rpm por 10 min) e ácido (0,5% (v/v) de ácido sulfúrico a 121ºC por 15 min) (carga de sólidos de 10% m/v). A hidrólise enzimática do material pré-tratado foi realizada utilizado o complexo enzimático CellicCtec® (60 FPU/$g_{biomassa\ seca}$, tampão citrato a 50 mM e pH 4,8) a 50ºC usando 150 rpm por 72h. Antes do processo de detoxificação, realizou-se um teste com a espécie de *Saccharomyces cerevisiae* para verificar se os compostos furfural (1 e 4g.L^{-1}) e ácido acético (1 e 5% v/v) exerciam significativa inibição na espécie testada. O processo de detoxificação avaliou a concentração de carvão ativado (1, 3 e 5% m/v) e o tempo do processo (30, 45 e 60 min) a 30 ºC, 150 rpm por 24 h. A composição lignocelulosica da biomassa *in natura* e pré-tratada (hidrotérmico e ácido) foi para celulose (36,7, 27,7 e 63,7%), hemicelulose (22,2, 4,4 e 12%) e lignina (21,2, 27,7 e 28,7%), respectivamente e com rendimento mássico em torno de 60%. A presença de furfural e ácido acético exibiu forte influência na espécie considerada, chegando a prejudicar em mais de 90% o consumo de açúcares no meio. O processo de destoxificação aumentou 13% a eficiência de fermentação para o hidrolisado obtido hidrotermicamente, enquanto que para o ácido não houve diferença significativa. Obtendo assim uma fermentação com maior eficiência, tecnicamente viável e menos poluente.
Palavras-chave: Matéria-prima lignocelulósica. Inibidores. Etanol. Produtos de degradação.

Introduction

Several studies have been conducted on the production of biofuels from renewable sources. In this context, lignocellulosic wastes composed of cellulose, hemicellulose and lignin are an important alternative (Shen & Agblevor, 2011; Santos-Rocha et al., 2016b; Ogando et al., 2016; Santos-Rocha et al., 2016a).

Sugarcane bagasse, an important residue from sugarcane processing, has been used for production of liquid biofuel in the past (Yu et al., 2013; Driemeier et al., 2015). However, sugars in this lignocellulosic waste are not readily available for fermentation because of the recalcitrance of this biomass. Therefore, to obtain biofuels from lignocellulosic materials additional steps such as pre-treatment and enzymatic hydrolysis (Santos-Rocha et al., 2017) are necessary. When a pre-treatment is applied (for example, steam explosion, liquid hot water or dilute acid), compounds that cause difficulties in later steps (hydrolysis and/or fermentation) are generated. These compounds are called inhibitors. These inhibitors interfere with fermentation efficiency because they are toxic to ethanol-producing microorganisms (Zeng et al., 2021).

Inhibitors can include lignin degradation products (a wide range of aromatic compounds), organic acids (acetic and formic), and furan derivatives (hydroxymethylfurfural and furfural) (Deng & Aita, 2018; Gurram & Menkhaus, 2014; Cardona et al., 2015). For example, the degradation of furfural is an aldehyde-alcohol transformation reaction, which includes furfuralcohol and furoic acid (Sun et al., 2020). To reduce the inhibitory effect of these compounds, the procedure of washing the biomass after pre-treatment is most commonly used, thus using a reasonable amount of water, causing the production of another effluent and the probable loss of sugars found in the biomass (Pan et al., 2019; Fernández-Delgado et al., 2019).

Several detoxification methods, such as the removal of inhibitors from lignocellulosic liquors, have been used to increase their fermentability. The adsorption process using charcoal treatment can achieve a high fermentation efficiency of the saccharified liquor by yeasts (Kim et al., 2011; Behera et al., 2014; Liu et al., 2015).

This study aimed to produce cellulosic ethanol by fermentation with S. cerevisiae from sugarcane bagasse hydrolysate, which was obtained after hydrothermal and acid pre-treatment and sequentially subjected to enzymatic hydrolysis and detoxification before the fermentation process, in order to remove inhibitors and obtain greater fermentation efficiency.

Materials and Methods

Raw material

Sugarcane bagasse was provided by the Coruripe Mill (Coruripe, Alagoas, Brazil). This feedstock was dried at room temperature until 10% of its moisture content in biomass was obtained. Then, it was milled in a Willey type mill to a particle size of 30 mesh, placed in plastic bags, and stored in a freezer (-8 °C) to prevent contamination.

Hydrothermal pre-treatment

Hydrothermal pre-treatment was carried out in a 5.5L stainless steel reactor (model 4584, Parr Instrument Company, Moline, IL, USA). Sugarcane bagasse was mixed with distilled water at a solid/liquid ratio of 1:10 (w/v) (10% of solid loading) inside the reactor. The reaction occurred under 195 °C for 10 min at 200 rpm. When the reaction was complete, the reactor was cooled to 40 °C

and the solids were filtered from the liquids. through the solid was then washed with water to remove dissolved contents until a neutral pH was reached.

Dilute sulfuric acid pre-treatment

Acidic pre-treatment with sulfuric acid solution (0.5%, v/v), at a solid to liquid ratio of 1:10 (w/w) (10% of solid loading), was carried out in an autoclave at 121 °C for 15 min. After returning to room temperature, the solids were filtered from the liquids. The solids were washed with water to remove dissolved content.

Chemical characterization of the biomass

Raw and pre-treated (hydrothermal and acidic pre-treatments) sugarcane bagasse were characterized with respect to their chemical composition, according to analytical procedures described by Sluiter et al. (2008), modified by Rocha et al. (1997) and validated by Gouveia et al. (2009).

Enzymatic hydrolysis of raw and pre-treated biomass

The enzymatic complex used was Cellic®CTec2, donated by Novozymes, Latin America (Araucária, Paraná, Brazil). This complex presented 245 $FPU.mL^{-1}$ (filter paper units) enzymatic activity (Ghose, 1987). Enzymatic hydrolysis was conducted with reaction volumes of 50 mL, 150 rpm, 72 h, at 50 °C in batches. Each batch was placed in sodium citrate buffer (50 mM, pH 4.8) using a solid loading of 10% (w/v) and an enzyme dosage of 60 $FPU.g^{-1}_{dry\ matter.}$ The hydrolysis efficiency was assessed by the release of Total Reducing Sugars (TRS), determined using the DNS method (Miller, 1959).

Testing the inhibitory effect of acetic acid and furfural on ethanol fermentation by S. cerevisiae

A 2^3 experimental design was used to evaluate the effect of acetic acid (1 (-1) and 5% (v/v) (+1)) and furfural (1 (-1) and 4 $g.L^{-1}$ (+1)) on ethanol fermentation at different yeast concentrations (0.2 (-1) and 1% (w/v) (+1)). A total of eight experiments were carried out in triplicate, as displayed in Table 1. Experiments were performed using a formulated synthetic hydrolysate and *Saccharomyces cerevisiae* yeast (Fermix®). Erlenmeyer flasks containing YPD medium (10 $g.L^{-1}$ yeast extract, 20 $g.L^{-1}$ peptone and 40 $g.L^{-1}$ glucose) were stirred in a shaker incubator at 30 °C, 150 rpm for 24 h. Sugar consumption was the response evaluated from the experiments. Sugar concentration before and after fermentation were measured using total reducing sugars (TRS), which were determined using the DNS method (Miller, 1959). Statistical analyses were performed using the STATISTICA® software (7.0 version).

Table 1
Sugar consumption during fermentation by S. cerevisiae

Assay	Yeast mass (%) (Dry weight) X1	Acetic acid content (%) X2	Furfural concentration ($g.L^{-1}$) X3	Sugar consumption (%)
1	0.2	1	1	30.0
2	1	1	1	42.5
3	0.2	5	1	16.3
4	1	5	1	12.5
5	0.2	1	4	22.5
6	1	1	4	30.0
7	0.2	5	4	11.3
8	1	5	4	5.0

Nota. *Experimental result deviations were lower than 3%, and for this reason were not represented in the Table.

Fermentation of bagasse liquor obtained by hydrothermal and acidic pre-treatment and enzymatic hydrolysis and the effect of detoxification step

After the previous study and confirmation of the inhibitory effect of acetic acid and furfural on the strain used in this study (S. cerevisiae), another experimental design was set up to evaluate a suitable detoxification condition that could improve ethanol yield. This experimental design involved the 2^2 experimental designs with three replications in the central point. A total of seven experiments were carried out in triplicate. Statistical analysis was performed using the STATISTICA® software (7.0 version). Hydrolysate samples from both hydrothermally pre-treated and dilute acid pre-treated sugarcane bagasse were subjected to detoxification using milled activated charcoal with a particle size of 2 mm. The variables of the detoxification process were the adsorbent (charcoal) concentration (1, 3, and 5% (w/v), -1, 0, and +1 conditions, respectively) and the time (30, 45, and 60 min, -1, 0, and +1 conditions, respectively). Fermentation was performed as described by Wolf (2011) in a shaker incubator at 30 °C and 150 rpm for 24 h. The TRS were measured using the DNS method (Miller, 1959). Ethanol concentration was determined using the dichromatic method (Association of Official Analytical Chemists [AOAC], 2005; Santos-Rocha et al., 2016b). The efficiency of the process (based on the response variable) was evaluated based on sugar consumption (%) in the medium (reducing sugars). Fermentation efficiency was calculated as follows:

$$Fermentation\ efficiency\ (\%) = \frac{Ethanol\ (\frac{g}{L})}{(TRS_{initial} - TRS_{final})(\frac{g}{L}).0.511}.100$$

where TRS is the total reducing sugar and 0.511 is the stoichiometric factor for the conversion of monosaccharides in ethanol.

Results and Discussion

Chemical characterization of the raw and pre-treated biomass

Raw and pre-treated lignocellulosic biomass were chemically characterized in terms of cellulose, hemicellulose, lignin, and ash contents, as shown in **Table 2**. Cellulose was found to be the main component. Significant removal of hemicellulosic fraction was reached after the hydrothermal (88.9%) and acidic (65.9%) pre-treatments. This behavior corroborates with that in previous studies, in which hydrothermal pre-treatment caused the auto-ionization of water, which acted as a catalyst, decreasing the pH of the medium and stimulating the depolymerization of hemicellulose (Ruiz et al., 2020). Studies in which time, temperature, and pH were varied, carried out by Chotirotsukon et al. (2021) demonstrated the removal of around 52.0% of hemicellulose (170 °C, 40 min, pH = 7.0) from sugarcane bagasse. Santos-Rocha et al. (2017) indicated that an increase in the reaction temperature contributed to a higher percentage of 86.9% hemicellulose (195 °C, 10 min, pH = 7.0) being removed. Similarly, the dissolution of hemicellulose is also characteristic of acidic pre-treatments. During thermochemical reactions, sulfuric acid acts as a catalyst, cleaving the glycosidic bonds and releasing hemicellulose monomers (Kuloyo et al., 2014; Pereira et al., 2016; Santos-Rocha et al., 2017). With respect to lignin content, a lower but important percentage was removed (26.9 for hydrothermal and 14.5% for acidic pre-treatments). It has been pointed out that in both pre-treatments, there is also a modest removal of the lignin fraction (Chotirotsukon et al., 2021).

Table 2
Chemical characterization of the raw sugarcane bagasse and in pre-treated biomass (in dry matter)

Components (%)	Raw material	Hydrothermal pre-treatment	Acidic pre-treatment
Cellulose	36.7 ± 0.2	60.0 ± 0.1	63.7 ± 0.1
Hemicellulose	22.2 ± 0.1	4.4 ± 0.1	12.0 ± 0.2
Total Lignin	21.2 ± 0.1	27.7 ± 2.1	28.7 ± 0.7
Ashes	13.8 ± 0.1	6.3 ± 0.3	2.3 ± 0.2
Mass Yield	--	55.96	63.13

Effect of inhibitor presence on S. cerevisiae's fermentation activity

Table 1 shows the results for each condition as indicated by the experimental design. Sugar consumption was achieved between the values 5.0-42.5%, showing clearly that the presence of these inhibitors affects the fermentation process by S. cerevisiae. Jönsson & Martín (2016), observed that these compounds (inhibitors) hinder growth and metabolism of the cell complex during fermentation. In assay 8, it was identified that the severity of their effect increases with their concentrations, by applying 1% yeast, 5% acetic acid and 4 $g.L^{-1}$ furfural.

Wikandari et al. (2010), observed that during fermentation with 1.5 g. L^{-1} of acetic acid, the activity of S. cerevisiae was completely inhibited. The same effect was observed when furfural concentration was above 1 $g.L^{-1}$ (Richardson et al., 2011). The effects of these parameters, acetic acid and furfural, were better visualized when there was a higher consumption of sugars, in Experiment 2, where the fermentation conditions had the lowest concentrations of inhibitors, in which 1% of yeast, 1% of acetic acid and 1 $g.L^{-1}$ of furfural were used.

To confirm the results obtained, a verification of the model was carried out. To do this, it was necessary to adapt our model to a linear, quadratic or even cubic model. A linear model was initially tested for simplicity **(Equation 2)**, using analysis of variance, which was verified through ANOVA **(Table 3)** showing good results with R^2 = 0.9993 and Y being related with sugar consumption (%) and *X1*, *X2* and *X3* for yeast concentration (%), acetic acid concentration (%) and furfural (g/L), respectively.

$$\boldsymbol{Y(X1,X2,X3)}\ (\%) = \mathbf{32.69 + 21.10.}\boldsymbol{X1}\mathbf{ - 2.95.}\boldsymbol{X2}\mathbf{ - 2,70.}\boldsymbol{X3}\mathbf{ - 4.70.}\boldsymbol{X1.X2} \qquad (2)$$

Table 3
ANOVA for the linear model of sugar consumption

Factor	Sum of Square (SS)	Degrees of freedom	Mean of Square	F value	p-level (p = 0.01)
1	12.251	1	12.251	15.682	0.15747
2	**798.001**	**1**	**798.001**	**1021.442**	**0.01991**
3	**132.031**	**1**	**132.031**	**169.000**	**0.04888**
1 by 2	**113.251**	**1**	**113.251**	**144.962**	**0.05275**
1 by 3	7.031	1	7.031	9.000	0.20483
2 by 3	7.031	1	7.031	9.000	0.20483
Error	0.781	1	0.781		
Total SS	1070.379	7			

The F test was performed according to Box and Wetz (1973), taking into account the appropriate degrees of freedom. The ratio between the mean square of the regression (MSR) and that of the residue (MSr) must be greater than the distribution point F in order to have a greater degree of reliability, if possible, ten times greater. Thus, MSR/MSr = 122.43 > (10 x $F_{4,11}$); $F_{4,11}$ = 3.36 (for 90% confidence), showed that we have a highly significant fit and that it fits the linear model well.

Figure 1A presents a Pareto chart, which graphically summarizes and displays the relative importance of a group of data. Effects which the rectangle are located on the right of the red line (p = 0.1), are statistically significant (Barros et al., 2001). We observed that the main effects are acetic acid content and furfural and the interaction between acetic acid and *S. cerevisiae* concentration. In **Figure 1B-C**, the interaction between furfural and acetic acid, as well as acetic acid and

yeast concentration, are shown (based on the relevance demonstrated in **Figure 1A** - Pareto chart). These indicated that better results were obtained when lower concentrations of acetic acid and furfural were used. In addition, lower yeast concentration was affected more by the presence and concentration of acetic acid, showing that higher yeast concentration can aid in minimizing the impact of acetic acid in the medium.

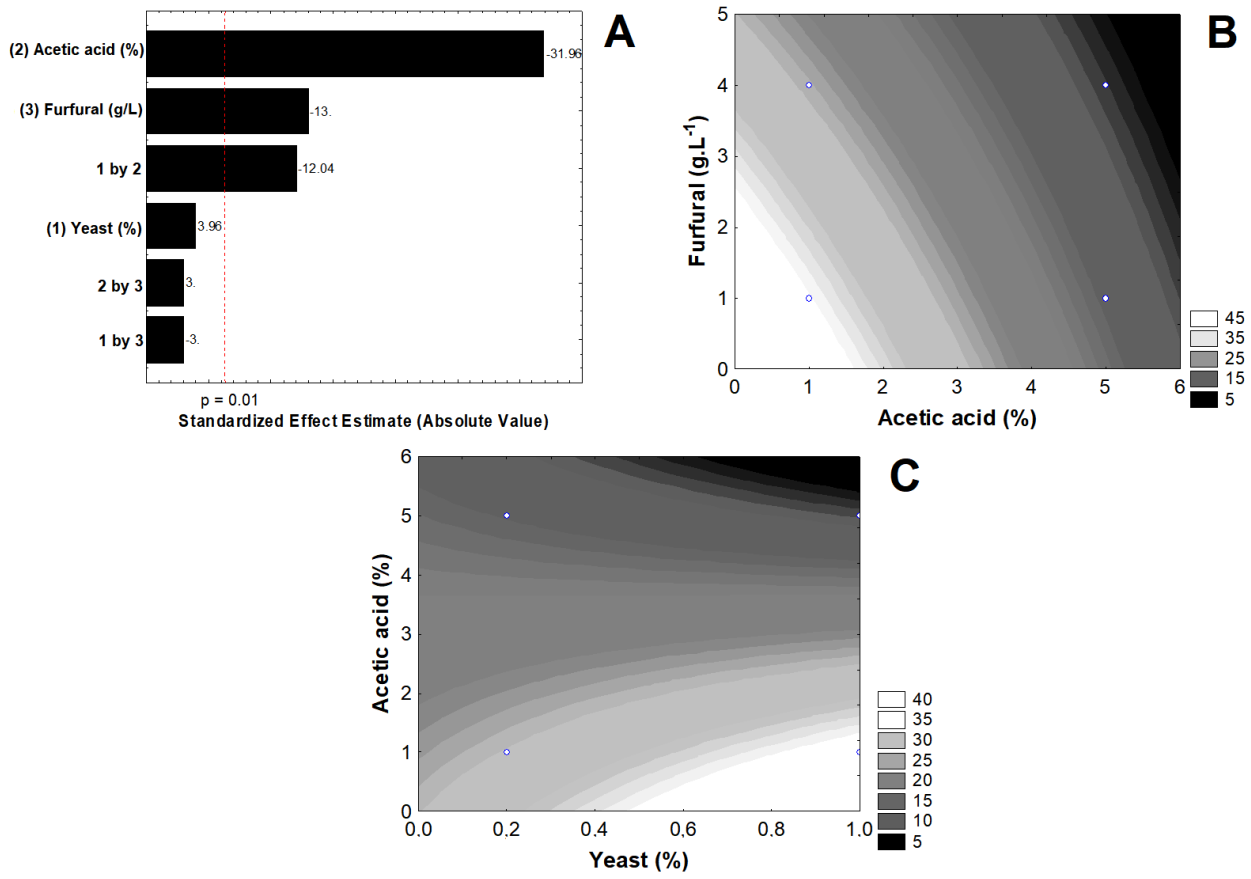

Figure 1. Figure showing the significance of the variables. The response variable is sugar consumption (%) in the medium represented by the numbers on the right of the graphs.
A) Pareto chart, B) Furfural Vs Acetic acid and C) Acetic acid Vs Yeast concentration.

Previous studies have shown that the presence of acetic acid and furfural affects the metabolization of sugars and, consequently the fermentation yield. Tian et al. (2009) utilizing 2 g.L^{-1} of *S. cerevisiae* obtained better fermentation efficiency when furfural was present up to 2 g.L^{-1}. The present study shows similarity with these prior studies with respect to the behaviour of *S. cerevisiae*, indicating a better performance in the presence of a greater amount of yeast. Also, Sarawan et al. (2019) verified that better fermentation results were obtained at lower concentration of acetic acid and furfural, 0.82 and 0.17 g. L^{-1}, respectively (fermentation with 1.2 g. L^{-1} of S. cerevisiae). Bezerra et al. (2020) cite that acetic

acid and furfural concentrations of 3 and 0.25 g. L^{-1}, generally have a toxic effect on ethanol fermentation. The verified citations present a margin of conditions close to those of our study. This can be observed in test 8, where the combination of maximum conditions of acetic acid and furfural has a negative effect on the number of sugars consumed. This makes it possible to analyze interference in the microbial growth rate and consequently the product metabolization (Oliva et al., 2006).

Detoxification and fermentation of sugarcane bagasse broth

The previous step showed that acetic acid and furfural were inhibitors of *S. cerevisiae's* fermentation of the lignocellulosic broth. Next, a detoxification step (adsorption on activated carbon) was applied to verify if the fermentation efficiency could be improved. However, it is important to mention that the TRS content could not be significantly reduced during this process.

Table 4 shows the results for each condition as indicated by the experimental design for the hydrothermal and acidic pre-treatments. The maximum recovery of sugars after detoxification was approximately 80% in both pre-treatments and for assay 1, which used 1% of adsorbent for 30 min. In other words, only about 20% of the sugars in the broth were lost during this process. This behavior can be attributed to adsorption by activated charcoal, which can remove both inhibitors and sugars. Contact time is a crucial variable that affects adsorption during detoxification processes, and there is a reaction time in which an equilibrium between the adsorbent (active carbon) and adsorbate (inhibitor compounds or sugars) is reached (Mussatto & Roberto, 2004). Villarreal et al. (2006) showed that an optimal pH (5.5) and contact time (60 min), were required for maximum removal of furfural (100%) from liquor from acid pre-treatment of eucalyptus biomass. For sugarcane bagasse, in the Pareto chat (graph not shown), the variable that showed significant influence was time (confidence level of 95%), i.e., higher the reaction time, 60 min, higher the sugar loss. **Figure 2** shows the effect of reaction time and adsorbent percentage on TRS recovery (g/L).

Table 4
Total reducing sugars (TRS) for hydrothermal and acid pre-treatment after detoxification process

Assay	Adsorbent (%)	Time (min)	TRS ($g.L^{-1}$) hydrothermal	TRS ($g.L^{-1}$) acidic
1	1	30	11.4 ± 0.3	34.3 ± 0.1
2	5	30	10.5 ± 0.4	30.9 ± 0.3
3	1	60	9.9 ± 0.3	29.9 ± 0.3
4	5	60	10.1 ± 0.1	24.6 ± 0.4
5	3	45	6.5 ± 0.3	32.9 ± 0.3
6	3	45	6.4 ± 0.3	32.5 ± 0.2
7	3	45	6.7 ± 0.2	31.2 ± 0.2

Nota. The initial concentration of sugars after enzymatic hydrolysis were 13.8 ± 0.2 and 41.8 ± 0.2 $g.L^{-1}$.

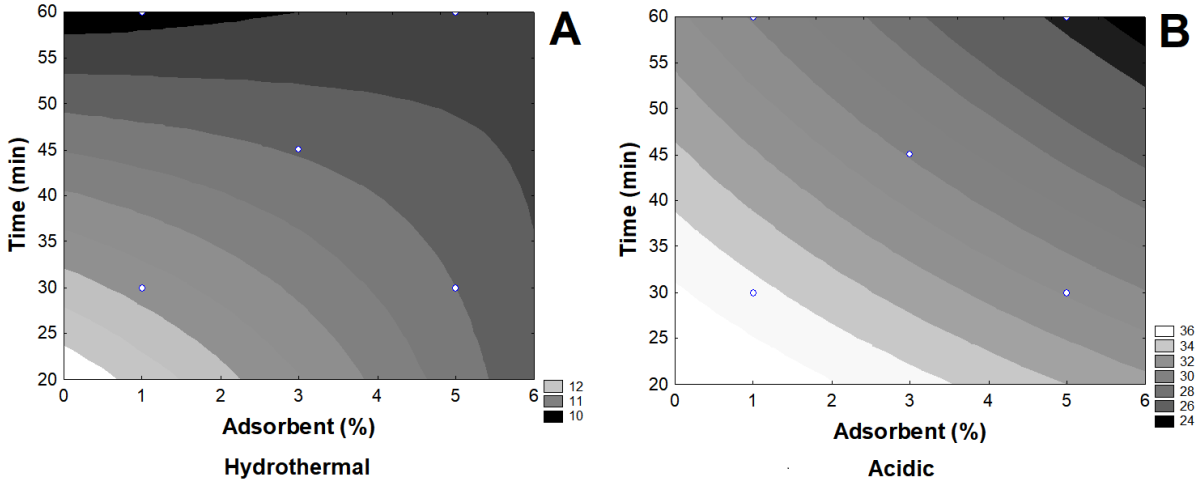

Figure 2. Relation between time and adsorbent dose to the total reducing sugars (TRS) (g/L) recovered from sugarcane hydrolysate A) for hydrothermal and B) for acidic pre-treatment.

Detoxification conditions used were based on literature. For example, Freitas et al. (2019) used 2% activated charcoal for the broth obtained from coconut husk pre-treated by acid and after 24 h, only 2% of the sugars were lost. Li et al. (2020) applied a detoxification process in a broth obtained from rice straw pre-treated with acid using 1% activated charcoal for 5 h, and a loss of 5% sugars was observed by them. Prasad et al., (2018), used a broth form corn straw treated by acid and conducted detoxification using 5% of activated charcoal for 30 min, to obtain a sugar loss of 15%, which is similar to our work.

Fermentation of non-detoxified and detoxified sugarcane bagasse broth

Finally, fermentation assays were carried out for sugarcane bagasse hydrolysates obtained after hydrothermal and acidic pre-treatments and enzymatic hydrolysis. Table 5 shows the results for the raw and detoxified hydrolysates using 1% of adsorbent and 30 min of process time.

Table 5
Ethanol concentration and fermentation efficiency obtained after the fermentation step

Assay	Bioethanol ($g.L^{-1}$)	Fermentation efficiency (%)
NHH*	7.17	75.33 ± 2.35[a]
DHH*	7.41	87.94 ± 1.56[b]
NHA*	4.92	70.41 ± 2.89[a]
DHA*	5.11	75.50 ± 2.39[a]

Nota. *NHA, non-detoxified hydrolysate from acid pre-treatment; DHA, detoxified hydrolysate from acid pre-treatment; NHH, non-detoxified hydrolysate from hydrothermal pre-treatment; DHH, detoxified hydrolysate from hydrothermal pre-treatment. Same letters represent no statistical difference with 90% confidence level ($p < 0.10$).

It is possible to verify an increase of 12.6% in fermentation efficiency for sugarcane bagasse hydrolysate obtained after hydrothermal pre-treatments and detoxification process. There was no significant difference between the detoxified and non-detoxified acidic hydrolysate.

Rasika et al. (2016) carried out fermentations using MDMC medium (with glucose and mannose), at 0.4 g. L^{-1} of acetic acid and 0.6 g. L^{-1} of furfural and obtained 94.7% (detoxified) and 56.13% (non-detoxified) of fermentation efficiency, showing the importance of detoxification in increasing ethanol yield. According to Freitas et al. (2019), detoxified liquor obtained from coconut husk after acidic pre-treatment increased fermentation efficiency from 60 to 84%.

A study carried out by Mussatto and Roberto (2004) indicated that the pH of the system influences the adsorption process of the inhibitor as a change in pH induces precipitation and causes instability of the toxic inhibitor compounds (Martinez et al., 2001). pH has an influence on furfural by altering the structural stability (Sahu et al., 2008). These studies suggest that, the low fermentative yield obtained from acid hydrolysate, before and after detoxification is due to the acid residues, that might have been retained from the acid pre-treatment. On the other hand, for hydrothermal hydrolysate, a simple process of detoxification with activated charcoal was shown to be significant.

The pre-treatment using sulfuric acid has been extensively studied because it de-structures lignocellulosic biomass (Behera et al., 2014). In addition, applying acidic solutions can generate more by-products, which could reduce the efficiency of consequent steps for obtaining biofuel. Hydrothermal pre-treatment is particularly significant in this context as it just makes use of hot-compressed water (Santos-Rocha et al., 2017).

Conclusions

These results emphasize that sugarcane bagasse is a potential lignocellulosic biomass for ethanol production. Acetic acid and furfural were toxic to the S. cerevisiae strain. A better performance was obtained for the liquor obtained from hydrothermally pre-treated biomass after detoxification with activated carbon. This study, shows that detoxification of compounds produced during pre-treatment and enzymatic hydrolysis of lignocellulosic biomass, which are inhibitors to fermentation by *S. cerevisiae,* improves the fermentation yield. It also showed that using hydrothermal pre-treatment, eliminates the washing step of pre-treated biomass, and eliminated the generation of new effluents.

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